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Antifungal Activities of a Wide Range of Medicinal Plants Extracts and Essential Oils Against *Scedosporium apiospermum* Isolates

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Abstract: We report the *in vitro* susceptibility of *Scedosporium apiospermum* species to a wide range of plant extracts. Twenty eight medicinal plant extracts and 21 essential oils of plants were tested against 60 isolates. Both groups of plant materials showed very promising activities against the isolates. This appears to be the first large scale testing of *Scedosporium apiospermum* species on a wide range of plant extracts and essential oils. In view of its widely reported resistance to conventional antifungals, more studies that will give further insight towards possible incorporation of these promising extracts in chemotherapy is strongly advocated.

Key words: *Scedosporium apiospermum* · Essential oils · Medicinal plants · Resistance · Antifungal

INTRODUCTION

There are a wide range of reports which indicate that some of the diseases caused by *Scedosporium species* were often fatal as some *Scedosporium* species are resistant to conventional antifungals such as amphotericin B, flucytosine, ketoconazole, miconazole, fluconazole and itraconazole [1-3]. This general observation has made the treatment of *Scedosporium* infections difficult. Some clinicians have combined amphotericin B therapy with flucytosine, fluconazole or itraconazole. Despite all these, mortality rate still remained high in most cases especially with disseminated infection [1]. It is inferred, therefore, that adequate treatment for *Scedosporium* infection is lacking and there is great need for new agents with favorable activity. In a recent review, Guarro *et al.* [4] just like other authors, similarly noted that numerous studies have proven that antifungal drugs such as amphotericin B, nystatin, itraconazole, flucytosine, fluconazole, terbinafine and ketoconazole show low *in vitro* activity against *S. apiospermum* [5-10]. There is therefore an urgent need for new agents with favourable activity against *Scedosporium* species. In the last couple of years, our laboratory has continued to search for possible antifungal principles from medicinal plants as possible alternatives. For instance, some plant materials were found to show good *in vitro* activity against dermtaophytes [11]. This study therefore tested the *in vitro* activity of a wide variety of plant extracts against *Scedosporium apiospermum* species isolated from clinical and environmental sources.

MATERIALS AND METHODS

Plant Materials and Their Identification: The plant materials used are shown below (Tables 1 and 2). Some of them were identified by Prof S.C Onyekwelu, a retired staff of the Department of Botany, UNN, while majority of them were identified by Mr. A.O. Ozioko, a retired laboratory staff of the Botany Department (with many years of training on plant taxonomy),University of Nigeria, Nsukka. The plants were obtained from different parts of Enugu and Ebonyi States in Nigeria. The plant materials were processed by separately drying them in the hot air oven at 50°C for 48 - 96 hours. The dried parts of the plants were subsequently reduced to fine powder using mortar and pestle. The essential oils of the plants listed below (Table 1) were also tested against the isolates and were obtained in a commercial form.

Ethanolic Extraction of Antimicrobial Substances from Plant Materials: The ethanolic extracts were obtained by macerating 100 grams of the plant power in 500ml of ethanol using a conical flask. All the flasks were sealed with paper foil to prevent loss of volatile solvent and left at room temperature for 2-4 days. At the end of this period, for each flask, the contents were filtered into a beaker and the residues discarded. Each filtrate was then concentrated by evaporating the solvent (ethanol). Percentage yield of the extract varied between 4.5 - 24.0%. The extracts were then put into sterile bottles, labelled accordingly and stored in the refrigerator while the test lasted.

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Table 1: List of medicinal plants of which essential oils were tested

	Essentials oils of:	Abreviation used
1	*Thymus vulgaris*	TV
2	*Syzygiun aromaticum*	SAr
3	*Salvia officinalis*	SO
4	*Rosa damascene*	RD
5	*Primula rosea*	PR
6	*Polianthus tuberose*	PT
7	*Pelargonum graveolns*	PGr
8	*Ocimum basilicum*	OB
9	*Michelia champaca*	MC
10	*Marjorana hortensis*	MH
11	*Lavandula officinalis*	LO
12	*Laurus nobilis*	LN
13	*Cymbogogon citrates*	CC
14	*Cinnamomum zeylanicum (leaf)*	CZl
15	*, ,, , (bark)*	CZb
16	*Cinnamomum cassia*	CCa
17	*Cedrus deodara*	CD
18	*Canaga odorata*	CO
19	*Barrintonia acatangula*	BA
20	*Daucus carota*	DC
21	*Piper nigrum*	PN

Table 2: List of medicinal plants/the parts used and percentage extract yield

	Medicinal plants tested	Plant part used	Abreviation used	% Extract yield
1	*Morinda lucida*	Leaf	M1	14.0
2	,,	Stem bark	M2	18.5
3	,,	Root	M3	14.5
4	*Zapotica portericensis*	Leaf	Z1	6.0
5	,,	Stem bark	Z2	4.5
6	,,	Root	Z3	24.00
7	*Napoleona imperialis*	Seeds	N4	14.00
8	*Chrysophyllum albidum*	Seeds	CA4	10.5
9	*Trema guineensis*	Leaf	T1	6.0
10	, ,	Stem	T2	8.50
11	, ,	Root	T3	10.50
12	*Cissus quadrangularis*	Leaf	C1	15.50
13	, ,	Stem	C2	11.00
14	, ,	Root	C3	13.50
15	*Parkia clappertonia*	Leaf	P1	20.00
16	, ,	Stem bark	P2	19.00
17	*Azadirchta indica*	Leaf	A1	24.00
18	, ,	Stem bark	A2	18.00
19	, ,	Root	A3	13.50
20	*Garcinia cola*	Seed	G4	18.00
21	*Picralima nitida*	Leaf	PN1	19.50
22	*Heinsa crinita*	Leaf	H1	18.50
23	*Senna alata*	Leaf	S1	12.50
24	*Piper guineensis*	NA	PG	17.50
25	*Zingiber officinalis*	NA	ZO	17.00
26	*Xylopia aethiopica*	NA	XA	20.00
27	*Averrhoa carabola*	Leaf	AC1	18.00
28	*Averrhoa carabola*	Leaf	AC2	9.00

Screening for Antimicrobial Activity

Preparation of Inocula: The isolates tested were subcultured on potato dextrose agar (PDA) incorporated with 0.5g/ml chloramphenicol for 5 to 7 days at 28° C. Conidia were collected with a cotton stick and suspended in sterile water. After the heavy particles have settled, the turbidity of the supernatants were measured spectrophotometrically at 530 nm and the

transmission was adjusted to 68 - 70% and diluted 100- fold to yield an inoculum of 1 x 10^4 to 5 x 10^4 CFU/ml. The purity of each standardized inoculum was tested by streaking a loopful of each suspension onto SDA plates.

Determination of Minimum Inhibitory Concentration (MIC) by Cup-plate Method: This was carried out as described previously [11]. Briefly, 0.01ml of the appropriate suspension of *each* isolate to be tested was thoroughly mixed with 20ml of warm sterile liquid Meuller Hinton agar and poured into the Petri dishes. The agar was left to solidify and 8mm bores were made on five positions on the seeded agar dish. The five wells were ascetically filled with 0.1ml of dimethylsulphoxide (DMSO) serially diluted extract. For the serial dilution, one gram of each plant extract was dissolved in 5ml of DMSO (to achieve 200mg/ml w/v concentration) and serially diluted to achieve two fold serial dilutions. The plates were incubated for 3 - 7 days at 28 °C. Two sets of controls were used. One control was the organism control and consisted of the seeded Petri dish central bore which had no plant material in it while the second control was the plant material control in which the plant materials were introduced into the wells of unseeded Petri dishes mainly to check for the sterility or possible growth coming from the plant materials. The entire cup-plate method screening test was done in triplicate. The highest dilution that showed clear inhibition zone is taken as the MIC.

Determination of Antimicrobial Activity of the Oils by Agar Well Diffusion Method: The agar well diffusion method was used. Zero point one ml of diluted inoculum (10^5 c.f.u. /ml) of test organism was spread on Meuller Hinton agar plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100 µl of essential oil of 100 mg/ml concentration and solvent blank (ethanol 70%). The plates were incubated for 5–6 days at 28°C. The antimicrobial activity was evaluated by measuring the zone of inhibition against test organism. The antibiotics, voriconazole and amphotericin B at 100 µ g/ml concentration each were used in the test system as positive controls.

Phytochemical Analysis of the Plant Materials: Basic phytochemical analysis which include detecting the presence of secondary constituents of plants such as alkaloids, flavonoids, glycosides,flavoniods,proteins, tannins, steroids and saponins in plant materials were done. These tests were carried out according to the methods described by Harbone [12] and later modified by Trease and Evans [13].

RESULTS

Extract Yield and Preliminary Antifungal Tests Results of Ethanolic Extracts of the Various Plant Extracts Against *Scedosporium apiospermum* Isolates: The percentage extract yield after the ethanolic extraction of the various plant materials is shown in Table 2. The yield varied according to the plant material involved. The leaves of *A. indica* and *Z. portericensis* gave the highest yield of 24 %, followed by *P. clappertonia* leaf and *X. aethiopica* extracts. The lowest yield of 4.5% was obtained from the stem-bark of *Z. portericensis*.

The ethanolic extracts of some plants investigated were not able to inhibit *Scedosporium* species. For instance *Z. portericensis* (leaf and stem-bark extract), *T. guineensis* (leaf extract), *C. quadrangularis* (leaf, root and stem-bark extracts) and *A. indica* stem-bark and root extracts were not able to inhibit any of the 24 isolates of *S. apiospermum* tested during the preliminary screening (Table 3).

Extracts from *T. guineensis* and *C. albidium* showed higher zone of inhibition on some isolates of *S. apiospermum* than other extracts. All the extracts of *M. lucida* (leaf, stem-bark and root) generally did better than other extracts tested in the preliminary inhibition because they individually inhibited various *S. apiospermum* tested in the study much more than the other plant extracts. *Z. portericensis* root extract and *Napoleona imperialis* seed extracts were also promising as they showed good inhibition on a greater number of *S. apiospermum* isolates.

Minimum Inhibitory Concentration (MIC) of Ethanolic Plant Extracts Against *Scedosporium Apiospermum* Species Tested: The results of the minimum inhibitory concentration (MIC) of promising plant extracts is shown in Table 4. Extracts from *Morinda lucida* and *Trema guineensis* (stem-bark and root) showed the least MIC values of 1.56mg/ml on some of the *S. apiopsermum* isolates tested. The MIC range for all the extracts tested varied between 1.56 and 100mg/ml. The three spices: *Piper guinnensis, Zingber officinalis* and *Xylopia aethiopica* generally showed good results compared to the others. *P. guineensis* for instance, showed good MIC results on 13 (56.5%) of the 23 isolates of

Table 3: Preliminary tests results of ethanol extracts of the various plant extracts on *S. apiospermum* isolates

	Plant extracts													
Isolates	M1	M2	M3	Z1	Z2	Z3	N4	CA4	T1	T2	T3	C1	C2	C3
SA1	+	++	+	-	-	++	+	-	-	+	-	-	-	-
SA11	-	-	-	-	-	-	++	+	-	++	+	-	-	-
SA80	+	+	+	-	-	++	+	+	-	-	-	-	-	-
SA92	-	++	+	-	-	-	-	++	-	+++	+	-	-	-
SA31	-	-	-	-	-	++	-	-	-	-	-	-	-	-
SA45	+	+	-	-	-	+	-	+++	-	++	+	-	-	-
SA35	-	-	+	-	-	-	+	-	-	+	+	-	-	-
SA55	-	-	++	-	-	-	+	-	-	-	+	-	-	-
SA61	-	+	-	-	-	-	-	-	-	-	-	-	-	-
SA66	+	+	+	-	-	+	+	++	-	++	+	-	-	-
SA68	+	+	++	-	-	-	+	+	-	+	+	-	-	-
SA75	-	-	-	-	-	-	+	++	-	+	+	-	-	-
SA77	-	-	+	-	-	+	-	-	-	-	-	-	-	-
SA79	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SA157	+	++	-	-	-	-	+	++	-	-	-	-	-	-
SA163	-	-	-	-	-	-	-	-	-	-	+	-	-	-
SA3	-	+	+	-	-	+	+	++	-	-	-	-	-	-
SA20	-	-	-	-	-	-	-	-	-	-	+	-	-	-
SA85	-	+	+	-	-	-	+	+	-	-	-	-	-	-
SA89	-	++	+	-	-	-	+	+	-	-	-	-	-	-
SA138	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SA148	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SA151	-	-	-	-	-	-	-	-	-	+	-	-	-	-

Key: -, no zone of inhibition; +, zone of inhibition from 1-8mm; ++, zone of inhibition from 9-13mm; +++, zone of inhibition from 14-19mm. Full names of plant extracts M1, M2, M3, Z1, Z2, Z3, N4, CA4, T1, T2, T3, C1, C2 and C3 are shown in Table 2.

Table 3: continued: Preliminary test results of ethanol extracts of the various plant extracts on *S. apiospermum* isolates

	Plant extracts													
Isolates	P1	P2	A1	A2	A3	G4	PN1	H1	S1	PG	ZO	XA	AC1	AC2
SA1	-	-	+	-	-	+	-	-	-	+	+	-	-	-
SA11	+	-	-	-	-	+	-	-	-	++	-	++	+	-
SA80	-	-	+	-	-	+	-	-	-	+	+	+	-	-
SA92	+	-	-	-	-	++	-	-	-	-	+	-	-	-
SA31	-	-	++	-	-	-	-	-	-	++	+	-	-	-
SA45	+	-	-	-	-	-	-	-	-	+	-	-	-	-
SA35	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SA55	+	-	-	-	-	+	-	-	-	+	-	-	-	-
SA61	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SA66	+	-	-	-	-	+	-	-	-	+	-	-	-	-
SA68	+	-	+	-	-	-	-	-	-	+	+	++	-	-
SA75	-	-	-	-	-	-	-	-	-	-	+	-	-	-
SA77	+	-	-	-	-	-	-	-	-	+	-	-	-	-
SA79	-	-	+	-	-	-	-	-	-	+	-	+	-	-
SA157	++	-	-	-	-	+	-	-	-	+	-	+	-	-
SA163	-	-	-	-	-	-	-	-	-	++	-	+	-	-
SA3	+	-	-	-	-	-	-	-	-	-	+	+	-	-
SA20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SA85	+	-	+	-	-	+	-	-	-	-	+	-	-	-
SA89	++	-	-	-	-	+	-	-	-	-	+	+	-	-
SA138	-	-	-	-	-	++	-	-	-	+	-	-	-	-
SA148	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SA151	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: -, no zone of inhibition;+, zone of inhibition from 1-8mm; ++, zone of inhibition from 9-13mm; +++, zone of inhibition from 14-19mm. Full names of plant extracts P1, P2, P3, A1, A2, A3, G4, PN1,H1, S1, PG, ZO,XA,AC1 and AC2 are as shown in Table 2.

Table 4: Minimum inhibitory concentration (MIC) (mg/ml) of ethanolic plant extracts on *S. apiospermum* isolates

	Plant extracts													
Isolates	M1	M2	M3	Z3	N4	CA4	T2	T3	P1	A1	G4	PG	ZO	XA
SA1	12.50	1.56	12.50	25.00	1.56	-	1.56	-	-	1.56	6.25	12.5	3.125	-
SA11	-	-	-	-	6.25	3.125	6.25	25.00	3.125	-	6.25	1.56	-	1.56
SA80	25.00	6.25	6.25	50.00	100.00	12.50	-	-	-	25.00	12.50	12.50	25.00	-
SA92	-	12.5	1.56	-	-	25.00	3.125	25.00	50.00	-	25.00	-	25.00	-
SA31	-	-	-	50.00	25.00	-	-	-	-	50.00	-	12.50	6.25	-
SA45	12.50	6.25	-	25.00	-	25.00	1.56	12.50	-	-	-	25.00	-	-
SA35	-	-	50.00	-	25.00	-	3.125	-	-	-	-	-	-	-
SA55	-	-	50.00	-	12.50	-	-	12.50	50.00	-	100.00	50.00	-	-
SA61	-	1.56	-	-	-	-	-	-	-	-	-	-	-	-
SA66	100	50.00	100.00	6.25	6.25	1.56	6.25	6.25	25.00	-	100.00	25.00	-	-
SA68	50	25.00	25.00	-	12.50	6.25	12.50	3.125	50.00	100.00	-	12.50	3.125	6.25
SA75	-	-	-	-	50.00	12.50	25.00	25.00	-	-	-	-	3.125	-
SA77	-	-	1.56	12.50	-	-	-	-	50.00	100.00	-	6.25	-	-
SA79	-	-	-	-	-	-	-	-	-	-	-	3.125	-	-
SA157	1.56	3.125	-	-	100.00	50.00	-	-	12.50	50.00	100.00	6.25	-	-
SA163	-	-	-	-	-	-	-	-	-	-	-	12.50	-	-
SA3	-	25.00	50.00	12.50	100.00	25.00	-	-	6.25	25.00	-	-	6.25	-
SA20	-	-	-	-	-	-	-	50.00	-	-	-	-	-	-
SA85	-	1.56	6.25	6.25	100.00	12.50	-	-	12.50	50.00	50.00	-	6.25	-
SA89	-	6.25	50.00	12.5	100.00	100.00	-	-	6.25	100.00	12.50	-	25.00	250
SA138	-	-	-	-	-	-	-	-	-	-	3.125	25.00	-	-
SA148	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SA151	-	50.00	-	-	-	-	25.00	-	-	-	-	-	-	-

Key; -,no activity, Full names of plant extracts M1, M2, M3, Z3, N4, CA4, T2, T3, P1, A1, G4, PG, ZO,XA are as shown in Table 2.

Table 5: Phytochemical analysis of the plant extracts with promising activities

	Phytochemical constituents of the plant extracts								
Plant extracts	Alkaloids	Flavonoids	Saponins	Tannins	Cyanogenetic glycosides	Anthracene glycosides	Cardiac glycosides	Steriod aglycone	Protein
M1	+	+	+	+	+	+	+	+	+
M2	+	+	+	+	+	+	+	+	+
M3	+	+	+	+	+	+	+	+	+
Z3	+	+	+	+	-	+	+	+	+
N4	-	+	+	+	-	-	+	+	+
CA4	+	+	+	+	-	-	+	+	+
T2	-	+	+	-	+	-	+	+	+
T3	-	+	+	+	+	-	+	+	+
P1	+	-	-	+	+	-	+	+	+
A1	+	+	+	-	-	+	+	+	+
G4	-	+	+	+	-	-	+	+	+
PG	-	-	+	-	+	+	+	+	+
ZO	+	+	-	+	-	+	+	+	+
XA	+	+	+	-	+	-	+	+	+
A2	+	+	+	-	+	+	+	+	+
PN1	+	+	+	+	+	+	+	+	+

Key: +, present; -, absent. Full names of plant extracts M1, M2, M3, Z3, N4, CA4, T2, T3, P1, A1, G4, PG, ZO, XA and PN1 are as shown in Table 2.

Table 6: Sensitivity of essential oils of some plant extracts on SA isolates *Scedosporium apiospermum* isolates

Oils Tested	SA1	SA11	SA80	SA92	SA31	SA45	SA35	SA55	SA61	SA66	SA68	SA75	SA77	SA79	SA157	SA163	SA3	SA20	SA85	SA89	SA138	SA141	SA151	CBS 100.26
TV	++	-	+	-	-	++	-	-	++	-	-	+	-	+	-	+	-	+	-	+	-	-	-	+
Sar	+	-	++	-	++	-	-	+	+	+	-	++	-	++	-	+++	-	++	+++	+	-	+	-	++
SO	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
RD	+++	+	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	-	+
PR	-	-	+	-	-	-	+++	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
PT	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	++	-	+	-	-	-	-
PGr	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-
OB	++	-	-	-	++	-	-	-	-	++	-	-	-	-	-	++	-	-	-	+	-	-	-	+
MC	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
MH	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
LO	-	+	-	-	-	-	++	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
LN	++	+	-	-	+	-	+	-	++	-	-	-	++	-	-	-	+	++	+	-	-	-	-	+
CC	+	-	++	+	-	+	+	-	-	-	-	++	-	+	++	-	-	+	-	++	+	+	-	+
CZl	+	++	+	-	+	-	+	++	-	+	+	-	++	++	-	++	++	-	-	-	-	++	-	++
CZb	-	+	-	+	-	+	+	-	++	-	+	-	-	+	-	++	+	+	-	++	-	+	-	+
CCa	+++	-	++	-	-	+++	-	++	-	+	+	+	-	++	-	+	-	+	+	-	+	+	-	-
CD	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
CO	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
BA	-	-	-	+	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
DC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
PN	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: -, no zone of inhibition; +, zone of inhibition from 1-8mm, ++, zone of inhibition from 9-13mm; +++, zone of inhibition from 14-19mm. Full names of essential oils tested TV, SAr, SO, RD, PR, PT, PGr, OB, MC, MH, LO, LN, CC, CZi, CZb, CCa, CD, CO, BA, DC and PN are as shown in Table 1

S. apiospermum whose MICs were determined. The MIC results did not follow any pattern in terms of the source or nature of the samples from where the organisms were recovered.

Phytochemical Analysis of Promising Plant Extracts: Table 5 shows the result of the phytochemical analysis of promising plant extracts. Cardiac glycosides, steroidal aglycone and protein were contained in all the extracts tested. The extracts varied in the other phytochemical constituents tested.

Cyanogenetic and anthracene glycosides, saponins, alkaloids, flavonoids and tannins were present in majority of the plant extracts as shown in Table 5. The plant materials tested were merely selected based on their antimicrobial activities against *Scedosporium* isolates.

Sensitivity of Essential Oils of Some Plant Extracts on *Scedosporium* Isolates: The result of the preliminary sensitivity tests of essential oils of some plant extracts on *S. apiospermum* is shown in Table 6. It shows that some of the tested essential oils demonstrated good preliminary activities against *Scedosporium apiospermum* isolates. Out of the 21 of them, at least one had activity on a single species of *S. apiospermum*. Oils from *Cinnamoum zeylanicum* and *Syzygium aromaticum* did better than others in inhibiting *Scedosporium apiospermum* isolates.

DISCUSSION

Some of the medicinal plants investigated in this study were able to yield more extract than some others. Majority of the plants were selected based on ethnopharmacologic and scientifically proven properties [11]. The results of the preliminary screening showed that while some plant extracts had inhibitory effects on some *Scedosporium* species, some did not show any activity. *M. lucida* extracts appears to exhibit the greatest inhibitory effect than the rest of the other plant extracts as both the leaf, stem-bark and root extracts showed activity against majority of the *S. apiospermum* strains. Out of the leaf, stem-bark and root extract of *Cissus, Zapotica* and *Averrhoa* screened, only the root extract of *Zapotica* and the stem-bark of *Averrhoa* showed preliminary activity on *S. apiospermum*. It would appear that so many plant extracts were investigated in this study. This was due to the initial unimpressive result that was obtained at the onset of the preliminary screening which necessitated the screening of more plant extracts.

The minimum inhibitory concentration of plant extracts that showed initial sensitivity against *S. apiospermum* is shown in Table 4. Extracts from *Morinda lucida* and *Trema guineensis* (stem-bark and root) showed the least MIC values of 1.56mg/ml on some of the *S. apiospermum* strains tested. In testing for the susceptibility of these plant extracts against

S. apiopermum, representative isolates recovered from a wide variety of non-clinical and clinical sources were used and their was no particular observation or trend shown with reference to the source of the isolates. In other words, the pattern of susceptibility to these ethanolic plant extracts did not vary according to the source or geographical location of the strains. This means that the susceptibility data was strain dependent. Some authors observed that different strains of filamentous fungi are variably susceptible to drugs even within the same species [14]. This finding is similar to the observation made while testing the other drugs for the same isolates in another study. Another interesting observation is the data obtained with the three spices used in the study namely: *Piper guinnensis, Zingber officinalis* and *Xylopia aethiopica*. Particularly worth mentioning is the ability of *P. guineensis* to show good inhibitory activities on 13 (56.5%) of the 23 strains of *S. apiospermum* tested. Several studies have tried to justify that spices have good antimicrobial activities [15]. These observations are in agreement with the findings in this study.

Considering the fact that *Scedosporium* species used in this study and several other studies have shown resistance to almost all conventional antifungals, some authors have suggested the need and indeed challenged pharmaceutical companies to come up with new drugs capable of tackling this fungal genus. In view of the fact that in this study, only crude ethanolic extracts were used, we considered a strong response to exist when the extracts produced an effect at concentrations of 25 mg/ml or less. Nevertheless, it might be an interesting thing to exploit some of the active constituents of these plants in drug discovery aimed at solving the problems caused by this multi-resistant species of *Scedosporium*. Another approach could be by using other solvents in carrying out the extraction from the plant materials as some authors have suggested that certain solvents extract more than others.

Compounds related to chemical groups several members of which have previously been associated with wide ranging antimicrobial and pharmacological activity *in vitro* and *in vivo* [16-17] were found to be contained in the plant materials tested as shown by the results of phytochemical studies. Some of these compounds like alkaloids, tannins and saponins from plants have previously been associated with antimicrobial activity [13,16]. Some of them kill microbial cells by precipitation of cell proteins and disruption of cell membrane integrity [18] while the alkaloids such as the protoberberine types achieve this objective by their ability to bind to microbial deoxyribonucleic acid of the target cell by intercalation of their quaternary cation [19]. Also more than one target organelles have been proposed as sites of action for some alkaloids [20-21]. Antimicrobial action by an alkaloid can be initiated at the cell-membrane and cell-walls with the subsequent loss of the normal membrane permeability function. Small molecules (ions, amino acids, sugars) would first leak out from the cells. Next, the loss of small molecules would greatly decrease cellular metabolism including energy metabolism, active transport and nucleic acid and protein synthesis. This would in itself be lethal since energy metabolism and synthetic activity would be required for recovery. With membrane barrier destroyed and the ionic strength of the cell reduced, the alkaloids have been established to convert DNA to complex I *in vitro*. It is this first complex that leads to profound alteration in DNA conformation resulting in an unwinding of covalently closed circular DNA, similar in geometry to that brought about by mutagenic intercalating dyes such as ethidium bromide [22]. For example, extracts from *M. lucida, C.albidium, P.clappertonia, A.indica,P. nitida* and the three spices investigated which contain alkaloids, in agreement with the findings reported from other alkaloid containing plants [16] demonstrated good antifungal activities against the test organisms. Other phytochemical constituents such as saponins, tannins, steroids, glycosides, flavonoids and the others may have individually or in combination with one another been responsible for the antifungal activity from the other species of plants tested which did not contain alkaloids. It is therefore difficult with the information available, to attribute their antimicrobial activity to any specific group of chemical components.

Essential oils were obtained from 21 species of different plants and investigated in the study. Essential oils have been known to show good antifungal activity against a wide range of fungi [23]. The low molecular weight of oils combined with pronounced lipophilic tendencies, allow them to penetrate cell membrane more quickly than other substances. This amazing ability of essential oils to penetrate tissue has been proven repeatedly in scientific experiments [14]. Essential oils penetrate tissue roughly 100 times faster than water and 10,000 times faster than salts. In this study, it was observed that some of the tested essential oils demonstrated good preliminary activities against

Scedosporium isolates. In fact out of the 21 of them, at least one had activity on a single species of *S.apiospermum*. Oils from *Cinnamoum zeylanicum* and *Syzygium aromaticum* did better than others in inhibiting *Scedosporium* isolates. It will be worth trying in future to ascertain the MIC of these oils on these isolates. This could provide more useful information on further exploitation of these oils in drug production aimed at combating the resistance shown by *Scedosporium* species.

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